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Archaeometric investigations on naturally and thermally-aged iron-gall inks using different tannin sources

Research Article

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Abstract: This paper investigates the behavior of paper strips containing iron-gall inks prepared with tannins from different sources, subjected to natural and thermally-induced aging. Results indicate that inks containing initial concentrations of ferrous sulphate ranging from 0.2 to 10.0 g are amenable to treatment with calcium phytate, and thata good correlation exists between the recovery of excess iron and the initial concentration. Infrared spectra showed an absorption band at 1,750 cm^{-1}, typical of esther, solely in the samples prepared with a condensed tannin. The condensed nature of this tannin produced a different oxidation pattern, with iron removal inferior to those observed from inks produced with hydrolisable tannins. When tannic acid was used ferrous iron removals ranged from 0.050 to 1.800 g, decreasing to 0.5 g in the presence of copper; the same behavior was observed for the remaining hydrolisable tannins, with a lower recovery from the condensed tannin. The adopted natural aging procedure released a higher amount of ferrous iron compared to ASTM thermal aging. This was probably due to the marked effect of humidity, not considered in the thermal procedure. A series of archaeometric possibilities were used to help elucidate the degradation of cellulose strips impregnated with iron-gall inks.

Keywords: *Archaeometry • Iron-gall ink • X-ray fluorescence • Infrared spectrometry • Tannin sourc*

1. Introduction

Iron-gall inks are produced by the chemical reaction between tannic acid and ferrous sulphate with the formation of a dark pigment known as ferrogallotannate or ferrotannate. This reaction is chemically unstable, gradually changing its color as time passes, consequently damaging the paper support.

Iron-gall inks that exhibit a higher stability to oxidation are those produced at the stoichiometric ratio of 3:1 gallotannic acid to ferrous sulphate. The mixture becomes an ink after the addition of a water-soluble binder, gum arabic, that improves the viscosity of the produced ink . Tannic acid is present on galls, bark, leaves, roots and fruits of several plants; however, in the galls, its concentration is markedly superior. Depending

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on the source of the plant, galls can be amorphous, as in Japanese and Chinese galls, globular, as in British and American oak galls, or very small, as in Aleppo galls, typically from Turkey, the ones with the highest content of gallotannate.

Ferrous sulphate is introduced in the formulation of the ink to color the product that is formed. During certain periods, copper sulphate was used instead of ferrous sulphate, however, with a markedly reduced contribution for this property.

After exposure of the ink to oxygen, ferric tannate is formed, a product that is not water soluble. In acidic environments hydrogen ions from gallic acid are not substituted by ferrous ion, due to the production of ferric tannate being markedly prevented at low pH values.

In a study of the chemical interactions in iron-gall inks, the role of the chemical compounds of the ink was investigated. Inks used in that case were composed of gallic acid, ferrous sulphate, and gum Arabic, and, in some cases, copper sulphate was also used. The authors observed that the formation of a new absorption band in the visible area could be used to calculate the rate of complex formation, parallel to the decrease in pH value and ferrous ion concentration. They also observed that gum arabic significantly enhanced the complex formation and its stability, and the addition of copper ions decreased the complex formation [1].

A study that investigated the degradation of iron-gall ink impregnated paper under various oxygen and humidity conditions, concluded that the oxygen concentration around 0.1% promoted cellulose depolymerization, whereas the relative humidity had no impact; the latter process wasregulated by oxidative reactions [2].

The use of infrared spectroscopy in paper samples and original documents was tested to analyze the original iron-gall inked manuscripts. Authors concluded that it was difficult to use this technique alone because of theseveral possible combinations of ingredients in the formulation of inks and their individual contributions on the degradation of the paper [3]. They observed that on laboratory probes the paper is close to the pure cellulose even if the paper is damaged. Diffuse reflectance microscopy performed directly on the sample was of limited use because of regular reflection. DRIFT spectroscopy offered a better reproducibility than transmission microscopy and was preferentially used for laboratory probes. Transmission microscopy was preferred for original samples, due to its non destructive nature. Authors also concluded that these techniques gave similar results on the evaluation of the contents in the original manuscripts and which presented great discrepancies between inked and non inked areas. The interpretation of the measured effects still faces a series of difficulties as the presence of the iron-gall complex cannot be detected solely by FTIR. The great differences observed in the spectra should also be attributed to the ink binding substance, and/or to the degradation state of cellulose in the paper.

Condensed tannins or proanthocyanidins, are polymeric flavonoids which constitute a group of metabolitesbased on a heterocyclic ring from phenylalanin and polyketide synthesis. The addition of a third phenolic group is still possible giving room to epigallocatechin and gallocatechin.

On the other hand, hydrolisable tannins are obtained from gallic acid, after sterification, producing hydrolisable tannins of higher complexity. Gallotannins, the simplest hydrolisable tannins, are polygalloyl esters of glucose which present several isomers with the same molecular weight of 940 g mol^{-1}.

Commercial tannic acid constitutes a mixture of gallotannins from sumac (*Rhus semialata*) galls (Chinese gallotannin), Allepo oak (*Quercus infectoria*) galls (Turkish gallotannin) and sumac leaves (*Rhus coriaria*). Due to its complexity it cannot be considered a standard molecule for comparison with vegetable tannins.

The amount of tannins present in *Acacia berlandierii*, *A. farnesiana, A. greggii* and *A. rigidula*, four common species found in the southwestern United States and northern Mexico, indicated that the bark and immature fruits had the highest percentage of tannins (5–15%), whereas the wood had a much lowercontent (less than 1%) [4].

Castanea sativa is a species of a flowering plant from the family Fagaceae, containing edible seeds known as sweet chestnut. Native to Southeastern Europe and Asia it is now widely dispersed throughout regions such as the lower Himalayas and other temperate parts of the Indian subcontinent. Century-old specimens may be found in Great Britain, western and southern Europe, Greece, Switzerland, Austria, Turkey, Portugal, France, Hungary, Italy, Slovenia, Slovakia, Serbia and Croatia. The concentration of tannins in the different parts of the plant have the following composition: bark (6.8%), wood (13.4%) and seed husks (10-13%) [5,6].

Caesalpinia spinosa (Molina) Kuntze, commonly known as Tara, is a small leguminous tree or thorny shrub native to Peru, cultivated as a source of tannins. The synonymies of the species include the following names: *Poinciana spinosa*, *Caesalpinia pectinata*, *Caesalpinia tara*, *Caesalpinia tinctoria*, *Coulteria tinctoria*, *Tara spinosa* and *Tara tinctoria*.

It is typically from Peru and can be found throughout northern, western and southern South America. It has been introduced in drier parts of Asia, the Middle East and Africa and has become naturalized in California.

It is a common source of hydrolysable tannins with a high content of gallic acid (53%) [7-11].

The genus *Acacia* is an old name adopted for a group of leguminous plants from five genera. Two of them – *Senegalia* and *Vachellia* – are the only ones found in Brazil, particularly *Senegalia,* with 52 identified species. The genus *Vachellia* includes only two typical species, *Acacia farnesiana* being the one widely distributed throughout the world.

Many *Senegalia* species are found in the Atlantic Forest and the Amazon rainforest; in the dry areas of the country, the most typical species that are found are *Acacia parviceps* (Speg.) Burk. e a *Acacia velutina* var. monadena Hassler.

Due to their wide distribution in tropical countries there are additionalspecies that are found as well. These are*Acacia bonariensis*, *Cassia ferruginea* (Schrad) Schrad ex DC, *Acacia jurema*, *Acacia farnesiana*, *Acacia cebil*, *Acacia decurrens*, *Acacia podalyraefolia* A. Cunn, *Acacia suma*, *Acacia nilotica*, *Acacia cultriformis* A. Cunn. ex G. Don, *Acacia melanoxylon*, *Acacia koa* (A. Gray), *Acacia cyanophylla*, *Acacia anegadensis* e *Acacia longifolia*, all of which are typically from Brazil.

Based on these considerations it can be easily concluded that a great number of combinations is made possible to use tannins in the preparation of iron-gall inks (concentration, source, type). The literature describes several classical and old recipes for the preparation of iron-gall inks, each one with its particular combination, depending on biological and chemical natures [12].

Ink oxidation occurs after several years of exposure to oxygen. So, it is easy to conclude that documents presently under clear chemical deterioration, were probably written hundreds of years ago. This way, it is essential to have an artificial and accelerated process for aging the documents in order to determine optimum treatment conditions.

The NISO standard requires paper to have an alkaline content equal to 2%, represented as calcium carbonate, as well as a maximum content of lignin equal to 1% to be adequate enough to allow the printing of paper. Even if paper meets these requirements, it stil may not be permanent if contaminated with copper or iron. These contaminants are potential accelerators of the degradation of paper in spite of the presence of the alkaline reserve [13].

In this context, one of the key problems with iron-gall ink is related to the oxidation of its components due to the natural aging of the documents, a difficult scenario to reproduce in laboratory conditions. During artificial or accelerated aging of a document, authors tried to expose it to extreme environmental conditions to obtain accelerated aging.

Several authors between 1950 and 2000 reviewed this topic and conducted tests. However, the authors observed the lack of standardization between the different procedures, usually based on thermal and relative humidity treatment against time [14].

A new method has been used to accelerate the aging of papers inside airtight glass tubes at 100°C for 5 days in order to gauge the retention of their original strength properties. This test was used as the ASTM standard to be adopted as the ISO standard [13].

In Brazil, the Museum of Astronomy and Related Sciences is responsible for the safekeeping of thousands of scientific documents. Many of them are unique samples of historic and scientific relevance, mainly written using iron-gall inks. If we consider that these documents: (a) come from different regions, thus, probably written using iron-gall inks produced from different tannin sources, with different gallic and gallotanic acid content; (b) were written in distinct periods, consequently, subjected to distinct oxidation and aging; (c) were maintained under distinct environmental conditions, consequently, with different degradation levels; and, mainly (d) were written using iron-gall inks prepared under non-stoichiometric conditions, it can be predicted that the oxidation of the components, as well as the heterogeneity of the recipes, and also the source of tannins used, are not known parameters. The objective of this work was to investigate the behavior of paper strips containing iron-gall inks prepared with tannins from different sources, with variable amounts of iron and subjected to natural and thermally induced aging. In order to achieve this objective, X-Ray fluorescence, Infrared spectroscopy were used, to have a combination of archaeometric possibilities to help elucidate the degradation of documents impregnated with iron-gall ink.

2. Experimental procedures

2.1. Iron-gall inks

Iron-gall inks were prepared with $FeSO_4{\bullet}7H_2O$ (Vetec, Brazil), tannic acid (A) ($C_{76}H_{52}O_{46}$) (Vetec, Brazil) or vegetable tannins from *Castanea sativa* (K), *Caesalpinia spinosa* (T) and *Acacia* sp. (W), provided by Tanac S.A./ Brazil, gum arabic and ethanol (Vetec, Brazil). In just one of the inks $CuSO_4{\bullet}5H_2O$ (Vetec, Brazil) was added. The combination of the reagents is described in Table 1; inks 1 and 2 have a stoichiometric relation between Fe(II) and tannic acid/vegetable tannin. The remaining ink preparations contain an excess of Fe(II), the preparation

Table 1. Iron-gall inks preparations used in the present work.

Ink	$FeSO_4 \cdot 7H_2O$ (g)	$CuSO_4 \cdot 5H_2O$ (g)	Tannic acid/vegetable tannin (g)	Gum arabic (g)	Ethanol (mL)
1	1.0	-	5.5	2.0	*
2	1.0	-	5.5	2.0	5.0
3	2.0	-	5.5	2.0	5.0
4	5.0	-	5.5	2.0	5.0
5	10.0	-	5.5	2.0	5.0
6	10.0	-	5.5	4.0	5.0
7	10.0	10.0	5.5	4.0	5.0

**Water as solvent*

of which was intended to simulate inks with an excess of iron and to observe the consequences of oxidation of this element to its ferric state, a fact that can be easily correlated with the degradation of printed documents.

The different iron-gall inks were prepared at room temperature, under atmospheric air for 24 h in order to help in the fixation of the components on paper strips and the strenghtening of the color after the formation of the gallotanic complex. After preparation, inks were kept in amber sealed flasks at room temperature. Several Whatman paper strips (6×1 cm) were immersed in the different flasks for 5 min. Strips were then dried at room temperature and left standing for 6 months at 30±4°C and 65±20% relative humidity, to simulate the natural aging of the paper. Simultaneously, a second group of paper strips were subjected to the same processing, however, under accelerated aging for 5 days at 100°C, inside glass tubes, according to the procedure adopted as the ASTM standard [13].

2.2. Infrared spectroscopy of tanic acid and vegetable tannins used for the preparation of iron-gall inks

In order to elucidate structural changes due to the formation of gallotanic complex, infrared spectroscopy (IV Nicolet Magna IR 560) were used to investigate vegetable tannins. Data were collected at 0.5 cm^{-1} intervals, with a resolution of 4.0 cm^{-1} with 64 data registrations. Spectra were obtained between 4000 and 400 cm^{-1} (Mid-IR) with the use of CsI tablets. The objective of this step of the investigation was to evaluate the distinct tannins and tannic acid in solid state and to obtain the information about the structures of condensed and hydrolisable molecules.

2.3. Treatment of iron-gall inks with calcium phytate for excess iron removal

Based on a previous work published in the literature, a 50% phytic acid solution (Aldrich, USA) was prepared, with the addition of the reagent (1.75 mL) and calcium carbonate (0.44 g) (Vetec, Brazil) [15]. After the reaction, the solution was added to deionized water (1 L) and ammonium hydroxide was added until the pH value reached 5.5-5.8. Before the immersion of the iron-gall ink impregnated strips into the calcium phytate solution, ethyl alcohol solution was added to decrease the strength of the paper strips. The prepared calcium phytate solution was distributed in several glass tubes of 25 mL each. Paper strips were immersed in the solution for 15 min at room temperature without agitation. Strips were then removed from the solution and left to dry at room temperature. The phytate solution used to wash and recover the residual iron from the strips was semiquantitatively evaluated for its iron content using iron-detection strips (Macherey-Nagel). The washing procedure was made by immersing the strips into the phytate solution until the iron content in the solution was stabilized. After washing the strips in the phytate solution, they were immersed in deionized water for 10 min to prevent crystals from formingduring drying. X-ray analysis was performed, as described below; secondly, two consecutive washings with phytate solution were done followed by a second X-ray analysis. This procedure was used for both the naturally and thermally aged inks.

2.4. X-ray fluorescence of paper strips containing iron-gall inks

As previously described, iron-gall ink X-ray determinations were performed (Dispersive energy X-Ray fluorescence spectrophotometer, model EDX 700 HS, Shimadzu) in the strips impregnated with iron-gall ink at several iron concentrations, before and after chemical treatments with calcium phytate,. These samples were the same as described in Table 1. The different spectra were used to quantify metal concentrations in the different ink formulations, before and after chemical treatment with calcium

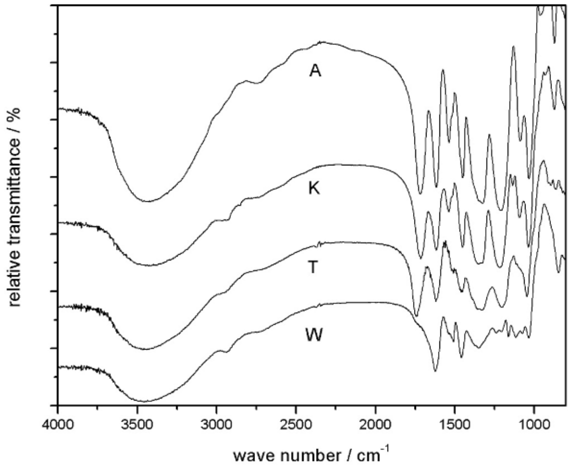

Figure 1. Infrared spectra of vegetable tannins and tannic acid used for the preparation of iron-gall inks.

phytate solution and also after natural and thermal accelerated aging, in order to check the stabilization of the ink. This would explain the possible changes in the chemical composition of the inks, corroborating the need for chemical treatment to remove the excess iron in the paper strips.

3. Results and discussion

3.1. Iron-gall inks

The prepared inks presented no differences among each other in respect to apparent viscosity, homogeneity and color immediatelyafter preparation. However, after a few weeks visible changes were noticed after atmospheric exposure andimpregnation of the paper strips, irrespective of the amount of ferrous salt and tannin type (or tannic acid) used. No signs of iron oxidation were observed after the six month natural aging process at 30±4°C and 65±20% relative humidity. Contrary, the set of inks thermally aged showed slight colordifferences as follows: inks prepared with the use of the vegetable tannins and tannic acid started to present oxidation, at different extents.Lnks prepared using Tara tannin (T) presented the lowest degree of oxidation, partially observed in the border of the paper strips, easily observable in the areas with

the presence and absence of the ink. It could also be observed that this oxidation tookplace stronger from inks 1 to 7, corroborating that increasing concentrations of ferrous iron in the ink lead to increasing oxidation patterns. This observation could be seen as well in the other inks prepared with Tannin K as well as with Tannic Acid (AT). Tannin K and Tannic Acid based inks presented lower oxidation patterns when compared to Tannin T.

Weibull tannin (W), on the other hand, presented a markedly distinct behavior in relation to oxidation. Even though oxidation increased from ink 1 to 7, iron oxidation was not restricted to the border of the inked area. The whole area of the paper strips showed that the iron present was completely oxidised. This behavior was observed solely in the Tannin W, the only condensed tannin. This fact corroborates the idea of investigating the different infrared spectra of the tannins to explain the amenability to the observed oxidation in some of the iron-gall ink preparations.

3.2. Infrared spectroscopy of tannic acid and vegetable tannins

The infrared spectra of the distinct tannins and tannic acid are presented in Fig. 1. The four infrared spectra of the vegetable tannins and tannic acid seem to have similarities.. Characteristic bands were observed around 3,500 cm^{-1}, represented by a strong and wide band, resulting from the polymeric association attributed to v (OH). Also, around 1,600 cm^{-1} a typical band related to the presence v (C=C) of aromatic rings, and an intense band around 1,750 cm^{-1} that can be attributed to the presence of esther v (C=O) are observed. In this particular case, it can be seen that this band is lacking in the infrared spectrum of the Tannin W. It can explain the fact that this missing compound in Tannin W is probably the source for its distinct behavior in comparison to the remaining tannins tested, all hydrolisable tannins, differently from Tannin W, that is a condensed tannin. This can be concluded, as the characteristic esther band was not observed in the spectrum of the Tannin W, as esther groups are only present in hidrolysable tannins.

The chemical species from the tannins involved in the formation of complex molecules during the production of iron-gall inks, is the gallotanic acid, a substance obtained from the hydrolisis of tannic acid. This tannic acid corresponds to a mixture of gallotannins from different vegetable sources.

During the production of iron-gall inks, several recipes were tested across the centuries, and in order to characterize the different inks, the literature focused on the substances that produced color, resulting from the reaction of ferrous sulphate and gallotanic acid.When combined in solution, gallotanic acid and ferrous sulphate tend to form a soluble and colorless complex molecule. Associated with this process is the recombination of sulphate ions that lead to the formation of sulphuric acid [16].

In the folowing step the complex formed is oxidised to form an additional complex between gallotanic acid and ferric iron. This solube product is black, the color of which tends to intensify after oxidation and its exposure to air.

Chemically, the formation of color occurs when ferrous iron, from ferrous sulphate, complexed by gallic and gallotanic acids are oxidized by ferric iron with the help of atmospheric oxygen, producing a blackish/blue product. This characteristic pattern of the iron-gall ink develops during the deposition of the ink on paper. This can be explained in terms of a transfer of reversible charge between the aromatic ring and the ferrous iron.
Once gallotanic and gallic acids are present in the hidrolysable tannins, it is suggested that the complex formed in the production of iron-gall ink can only be obtained from a hydrolysable vegetable tannin. However, there is a wide range of recipes available in the literature, as described in the historic recipes of this ink; this indicates that several documents written with iron-gall inks could have been prepared with condensed vegetable tannins, and, consequently, the formation of the complex could not have happened adequately. Other types of complexes could have been formed involving ferric iron, however, not suitable for the purpose of using the mixture as ink.

Manuscripts from the 19th century, consisting of paper pages and leather bookbinding, were investigated with the use of analytical techniques to identify the components of the manuscript and to explain its deterioration process [17]. Visual assessment, isolation and identification of fungi, pH measurements, and investigation of the surface morphology by scanning electron microscopy were used to explain paper and leather deterioration. Other analytical techniques were used to identify pigments, binder of pigments, ash, lignin, and the α-cellulose content of papers. The shrinkage temperature measurement was used to explain the deterioration process of leather. The results revealed that the ink used was a mixture of carbon with iron gall. The values of the shrinkage temperature and pH were lower than in normal conditions, indicating deterioration. *Aspergillus* sp., and *Penicillium* sp. were the most dominant fungi found on the manuscript. *Acacia arabica* was identified as the tanning material used with the bookbinding.

These conclusions corroborate the results obtained in the present paper, showing that *Acacia* tannin highly contributes to the deterioration of paper, due to the decreased content of complex between the condensed tannins and ferrous iron. This probably opens the possibility for a more pronounced fungal contamination due to the fragile nature of the paper impregnated with iron-gall inks produced with this type of vegetable tannins.

Some authors observed that both microbial and chemical factors may be the causes of ink eating, due to deteriogens produced during fungal action on cellulose fibers. However, the authors stress that biological deteriogens seem to be less aggressive than the deleterious effects caused by the catalytic action of ferrous iron [18].

In order to characterize three ink typologies, distinguishing them not only visually but also according to their chemical features, some researchers demonstrated that the use of iron–gall ink continued until the end of the19th century: in the chromatogram the characteristic peak due to the presence of permethylated gallic acid was found. The spread of the ink around printed characters was considerable and is a likely cause of accelerated paper aging [19].

In addition, some researchers investigated a set of inks, based on some ancient Armenian recipes and analyzed the different inks after air-drying them to obtain solid samples. A simple analytical procedure was adopted which excludes any treatment of the samples and consists in introducing the solid sample directly into the injector of the furnace pyrolyzer. Comparing the pyrograms, it was possible to discriminate fragments derived from ink, from that formed by the decomposition of paper affected by the ink [20].

The technique of FTIR studies tannin treated parchment, where different methods and concentrations are used, together with a tannic acid reference spectrum [21]. The characteristic tannin CO stretching at 1,710 cm^{-1} appears as a shoulder when parchment was treated with a low tannin concentration. However, it is well resolved in the spectrum for higher tannin concentrations. The tannin ring C-C stretching at 1,610 cm^{-1} superimposes the peak due to the presence of amide, causing a banc broadening and a shift of the peak centre that is better detected on parchments where enzymatic attack was used as treatment. Parchment FTIR modes

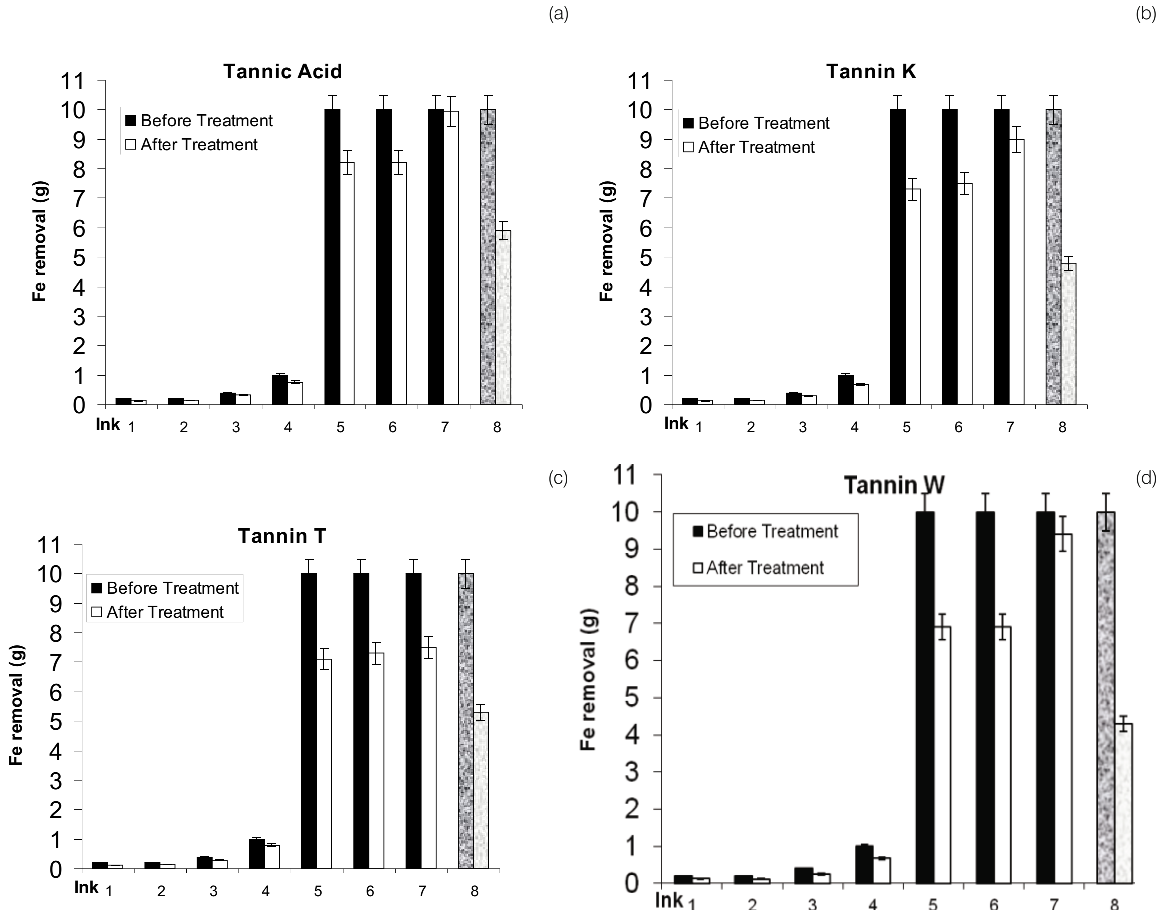

Figure 2. Iron removal from naturally aged iron-gall inks after treatment with calcium phytate solution. (Bars represented as Ink 8 correspond to percent copper removal from Ink 7). Inks prepared using: (a) Tannic Acid; (b) Tannin K; (c) Tannin T; and, (d) Tannin W.

are less intense at the1,000–1,300 cm^{-1} rang, which is a remarkable diagnostic region: tannin peak at 1,030 cm^{-1} (superimposition between stretching modes of the esther –C–O–C– and the carboxylic C–O–) and around 1,200 cm^{-1} (superimposition between stretching mode of C–O–C and bending mode of C–H) emerge from the parchment features even at low tannin concentrations.

Although distinct from the objective of the present paper, it can be concluded that tanning treatment affects the spectral features obtained through FTIR, and the vegetable tanning could be recognizable using this type of spectroscopy.

3.3. X-ray fluorescence of paper strips subjected to natural aging – Effect of phytate treatment

Fig. 2 presents the results obtained from the percent of iron removal, based on X-ray fluorescence results, before and after treatment with the calcium phytate solution for naturally aged paper strips containing different contents of iron-gall inks.

Irrespective of the tannin source, iron removal was observed for all the samples. The removal was more pronounced when Tannin W was used in the mixture. This indicates that the condensed Tannin W is more amenable to iron release, thus presenting a higher instability of the iron gall ink.

The ability of phytate to bind metals, particularly iron, demonstrates its highly antioxidant action. Calcium phytate inhibits the hydroxyl ion, mediated by ferrous ion, due to its chelating properties. This behavior turns the ion catalytically inactive. Beyond this observation, phytic acid markedly changes the iron redox potential, maintaining its ferric state and thus protecting against oxidative damages. Ferrous iron produces oxyradicals, while ferric iron is relatively inert [22].

The results of ferrous iron removal after treatment with calcium phytate, in naturally aged paper strips containing iron-gall inks, is presented in Table 2.

Table 2. Percent Fe(II) removal after treatment with calcium phytate solution (naturally aged paper strips containing iron-gall inks).

	% Fe(II) removal in the inks							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8***
Tannic Acid	32 (0.064g)**	25 (0.050g)	18 (0.082g)	24 (0.240g)	18 (1.800g)	18 (1.800g)	5 (0.500g)	41 (1.043g)
Tannin K	30 (0.060g)	26 (0.052g)	27 (0.108g)	32 (0.320g)	27 (2.700g)	25 (2.500g)	10 (1.000g)	52 (1.323g)
Tannin T	37 (0.074g)	27 (0.054g)	32 (0.128g)	21 (0.210g)	29 (2.900g)	27 (2.700g)	2.5 (0.250g)	47 (1.195g)
Tannin W	36 (0.072g)	40 (0.080g)	37 (0.148g)	32 (0.320g)	31 (3.100g)	31 (3.100g)	6 (0.600g)	57 (1.450g)

** Copper*
*** Results represent average results from 5 replicates with 5% error standard deviation*

After comparing the results obtained from Inks 1 and 2, these indicate that the presence of ethanol in the formulation slightly contributes to the stability of the ink, except when Tannin W is used. This is in close agreement to an old report that emphasized the need of “a pint wanting a quarter of wine” [23] and a recent report that suggests the use of “brandy to protect against freezing” [12].

These results showthat a comparison between Inks 2 to 5 indicate a close relationship between iron concentration during preparation and iron released after treatment. There is a linear correlation among these results, showing increasing and proportional release of ferrous iron as a function of initial ferrous iron concentration used in the formulation of the inks. A simple linear regression using the results presented in Table 2 (Inks 2 to 5) indicate that determination coefficients ranged from 0.8989 to 0.9292, depending on the source of tannin. It is important to mention that Tannin W presented the less favorable correlation between the results (determination coefficient equal to 0.8989), associated to the highest ferrous iron removals. These results corroborate the hypothesis that calcium phytate treatment to remove excess ferrous iron from iron-gall inks can be easily estimated, due to the linear behavior observed.

The effect of the addition of gum arabic, could be observed from the results obtained during iron removal in Ink 6; the addition of a higher amount of gum arabic, although contributing to the hardness of the ink, did not affect the stability of the ink, in relation to the iron release.

On the other hand, the presence of copper in combination with the iron in the ink, highly affected the removal of excess iron from the ink. It can be seen from the results obtained from Ink 7 that a much lower amount of iron was recovered from this ink, in comparison to Ink 6. This fact is compensated by the high removal of copper that was observed, irrespective of the source of tannin. Again, Tannin W released the highest levels of iron and copper, in comparison to the remaining tannins, indicating that condensed tannins tend to form unstable inks and so, contribute to a higher deterioration of printed documents.

Some authors observed in natural ecosystems the low decomposition of tannin rich leaves, a fact that can be partially attributed to the low levels of available metals, as chelation changes the redox potential of the metals, preventing its involvement in oxireduction reactions [24]. This way, chelating agents can be classified as Fenton-driven oxireduction reactions inhibitors. It is known that phenolic compounds are powerful metal chelating agents, decreasing their bioavailability, and this process can be easily compared to the mode of action of bacterial siderophores, phenolic molecules with a high iron uptake capacity [25,26]. Once chelated metals are no longer bioavailable, it can be stablished a correlation between tannin decomposition and the presence of metals. The efficiency of extraction of iron from model papers with different ligands at different concentrations and pH values, showed the best results were obtained with the use of a diethylenetriaminepentaacetic acid solution (0.005 mol L^{-1}), at pH 9.0, obtaining 97% of free iron extraction; however, authors observed a 64% removal of iron deposited in the form of ink [27].

Some investigators studied iron gall ink-induced corrosion of cellulose, proposing a combination of the complexing agent calcium phytate and calcium hydrogencarbonate in aqueous solution, obtaining excellent ferrous iron removal [28]. The authors also studied long term stability of the ink, after an aging step was performed after treatment, observing the preventive effect of the treatment.

In an investigation about iron gall ink-induced corrosion of cellulose, through the application of historic sample material, authors observed that, although the historic samples had been more severely oxidized than model papers, the inhibition of oxidation and hydrolysis by calcium phytate/hydrogen carbonate treatment was evident [29].

Other investigators tested the presence of transition metals in iron-gall inks with the purpose of studying

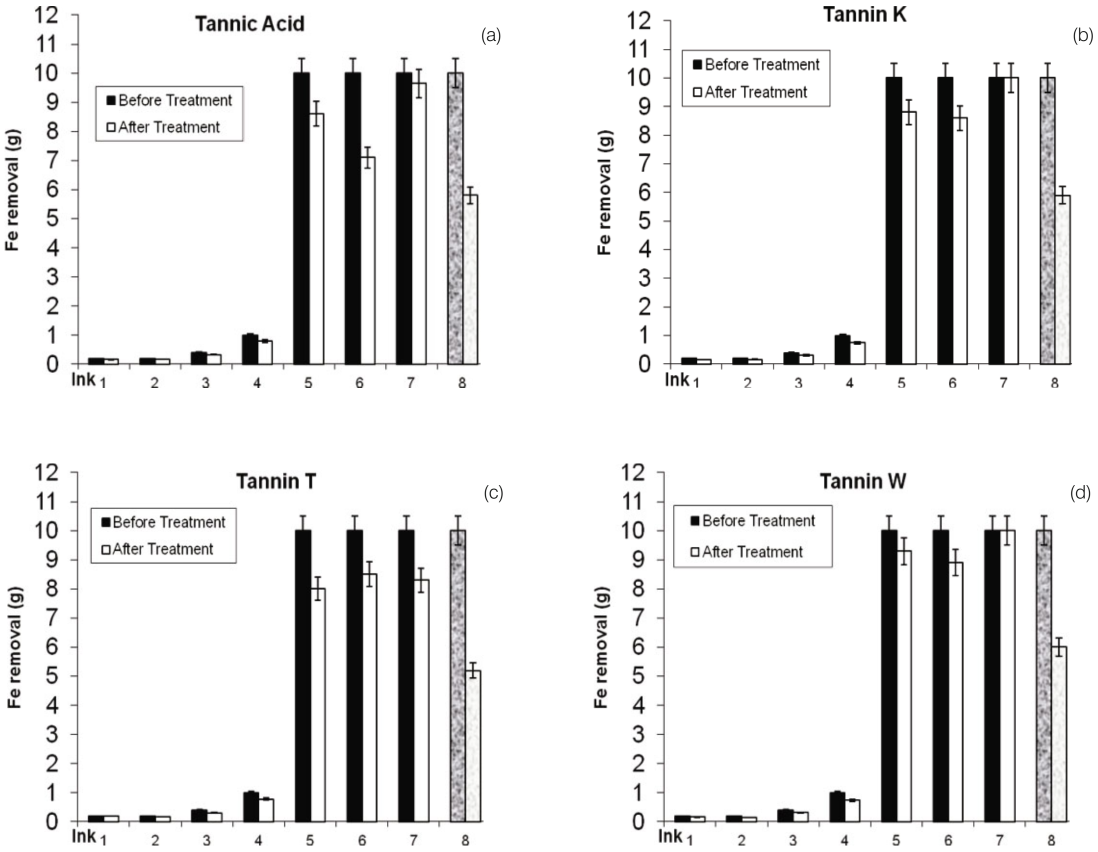

Figure 3. Iron removal from thermally aged iron-gall inks after treatment with calcium phytate solution. (Bars represented as Ink 8 correspond to percent copper removal from Ink 7). Inks prepared using: (a) Tannic Acid; (b) Tannin K; (c) Tannin T; and, (d) Tannin W.

Table 3. Percent Fe(II) removal after accelerated thermal aging, followed by treatment with calcium phytate solution (thermally aged paper strips containing iron-gall inks).

	% Fe(II) removal in the inks							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8***
Tannic Acid	16 (0.032g)	12 (0.024g)	20 (0.080g)	20 0.200g)	14 (1.400g)	29 (2.900g)	3.5 (0.350g)	42 (1.068g)
Tannin K	23 (0.046g)	17 (0.034g)	22 (0.088g)	25 (0.250g)	12 (1.200g)	14 (1.400g)	0 (0)	41 (1.043g)
Tannin T	0 (0)	12 (0.024g)	24 (0.048g)	22 (0.220g)	20 (2.000g)	15 (1.500g)	17 (1.700g)	48 (1.221g)
Tannin W	16 (0.032g)	27 (0.054g)	20 (0.040g)	26 (0.260g)	7 (0.700g)	11 (1.100g)	0 (0)	40 (1.017g)

**Copper*
*** Results represent average results from 5 replicates with 5% error standard deviation*

the catalysis promoted by these elements on cellulosic structures and its clear relation with deterioration against time [30].

3.4. X-ray fluorescence of paper strips subjected to thermal aging – effect of phytate treatment

Fig. 3 presents the results obtained from the percent of iron removalafter treatment with calcium phytate, for thermally aged paper strips.

Results from Fig. 3 indicate that ferrous iron removals followed the same pattern observed from the samples prepared after natural aging. However, it is clear that the amount of ferrous iron released was considerably lower. This is probably due to the absence of humidity, as predicted in the ISO standard procedure to age the samples. The deleterious effects of humidity on cellulosic fibers and its close relation to deterioration processes are widely known.

Analogously, Table 3 presents the results calculated of ferrous iron removal with calcium phytate after accelerated thermal aging. Results clearly indicate that the total mass of ferrous iron recovered after treatment is quite lower than the ones obtained with the same samples subjected to natural aging processes. Here, again, the amount of ferrous iron recovered from Tannin W samples is inferior to the ones obtained for the remaining samples with Tannic Acid, Tannin K and Tannin T. This is a clear indication that the condensend nature of Tannin W retains ferrous iron in its structure, thus preventing its recovery through chemical treatment. The consequences include the most fragile nature of the paper strips and higher oxidation rates, as ferrous iron is not easily removed. It also must be emphasized that when copper is present in the formulation of the ink, in combination with ferrous iron, it highly affects the recovery of ferrous iron (Inks 7 and 8). A mixed composition of ancient iron-gall ink recipes that usually included the addition of copper sulphate, in combination with or substitution of ferrous sulphate, requires a more detailed investigation.

It has also been observed that the addition of copper ions to the iron-gall ink solution decelerated the complex formation considerably, monitoring this decrease through UV-CIS spectra [1].

Again, a close correlation was observed in the present results: determination coefficients ranging from 0.8381 to 0.985 were observed through linear regression from the results obtained with inks 2 to 5. This indicates that a calcium phytate treatment can be a good alternative, stoichiometrically reliable to treat excess ferrous iron in iron-gall inks, at least for these conditions.

A comparison of degradation products from Whatman filter paper aged under humid and dry conditions were obtained, with the observation that glucose and xylose obtained from the hydrolytic degradation of cellulose and xylan constituted the main reaction products for papers at 90°C and 100% relative humidity [31]. Artificial aging performed at 90ºC and above, produced a mixture of products with a very little glucose content, demonstrating that hydrolysis of cellulose was not the dominant reaction under relatively dry aging.

Some investigators subjected a naturally aged paper to thermal aging from 50 to 95°C. They were convinced that there was no correlation between natural and accelerated aging and suggested that artificial aging should be at a temperature of 80°C and a relative humidity of 65% [32]. Later, another author [33] expressed concern regardingthe accelerated aging techniques, as he attempted without positive results to find reproducible degradation patterns with different papers.

The validity of accelerated aging techniques, as well as the use of Arrhenius plots for the aging of paper was also questioned [34]. The mechanism by which paper degrades at higher temperatures is different from the mechanism prevailing under ambient conditions. In this case, the author showed that each paper has a unique history that determines how that paper will age in the future, concluding that accelerated aging experiments cannot predict that pattern.

4. Conclusions

Iron-gall inks with excess ferrous iron oxidize differently, depending on the tannin source. Condensed tannins present higher oxidation rates, thus releasing less ferrous iron, amenable to be recovered with a calcium phytate solution.

Infrared spectra indicated that the condensed tannin presented distinct absorption bands in comparison to hydrolysable tannins.

X-ray fluorescence proved to be a useful tool for the estimation of percentage amounts of elements on paper strips impregnated with iron-gall inks, at different concentrations.

The percentage of ferrous iron recovered by calcium phytate solution was proportional to the initial amount of ferrous iron added during formulation, both in naturally and thermally aged samples.

The presence of copper, in combination with ferrous iron in the ink, highly compromises the recovery of iron, due to the preferential chelating properties of tannins to that element. This is an indication that treating original documents impregnated with iron-gall inks, containing simultaneously these elements, must be done with extreme care

The source of tannin used during ink preparation proved to be important. Condensed tannins were not desirable, due to their differentiated chelating nature.

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